

Deep-Sea Research II 57, 210–221 (2010)

ALEXANDRIUM MINUTUM RESTING CYST DISTRIBUTION DYNAMICS IN A CONFINED SITE

Sílvia Anglès¹, Antoni Jordi^{2,3}, Esther Garcés¹, Gotzon Basterretxea³, Albert Palanques¹

¹Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

²School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA

³IMEDEA (UIB-CSIC), Miquel Marqués 21, 07190 Esporles, Spain

ABSTRACT

The life cycle of the toxic dinoflagellate *Alexandrium minutum* consists of an asexual stage, characterized by motile vegetative cells, and a sexual stage, a resting cyst that once formed remains dormant in the sediment. Insight into the factors that determine the distribution and abundance of resting cysts is essential to understand the dynamics of the vegetative phase. In investigations carried out between January 2005 and January 2008 in Arenys de Mar harbor (northwestern Mediterranean Sea), the spatial and temporal distribution patterns of *A. minutum* resting cysts and of the sediments were studied during different bloom stages of the vegetative population. Maximum cyst abundance was recorded mainly in the innermost part of the harbor while the lowest abundance always occurred near the harbor entrance, consistent with the distribution of silt-clay sediment fractions. The tendency of cysts in sediments to increase after bloom periods was clearly associated with new cyst formation, while cyst abundance decreased during non-bloom periods. Exceptions to this trend were observed in stations dominated by the deposition of coarse sediments. High correlation between the presence of cysts and clays during non-bloom periods indicates that cysts behave as passive sediment particles and are influenced by the same hydrodynamic processes as clays. In Arenys de Mar, the main physical forcing affecting sediment resuspension is the seiche, which was studied using *in situ* measurements and numerical models to interpret the observed distribution patterns. During non-bloom periods, cyst losses were smaller when the seiche was more active and at the station where the seiche-induced current was larger. Thus, seiche-forced resuspension appears to reduce cyst losses by reallocating cysts back to the sediment surface such that their burial in the sediment is avoided. The observed vertical profiles of the cysts were consistent with this process.

RESUM

El cicle de vida de la dinoflagel·lada tòxica *Alexandrium minutum* es compon d'una fase asexual, caracteritzada per cèl·lules vegetatives mòbils, i una fase sexual, el cist de resistència, que una vegada format roman latent en el sediment. El coneixement dels factors que determinen la distribució i abundància de cists de resistència és fonamental per comprendre la dinàmica de la fase vegetativa. Es van estudiar els patrons de distribució espacial i temporal dels cists de resistència d'*A. minutum* i dels sediments durant diferents etapes de proliferació de la població vegetativa entre gener de 2005 i gener de 2008 al port d'Arenys de Mar (nord-oest del Mar Mediterrani). Les majors abundàncies de cists es van quantificar principalment en la part més interna del port, mentre que la menor abundància sempre es va produir prop de l'entrada del port, en concordança amb la distribució de les fraccions de sediments de llims i argiles. L'abundància dels cists en els sediments va augmentar després dels períodes de proliferació amb la formació de cists nous, mentre que l'abundància de cists va disminuir durant els períodes d'absència de proliferació vegetativa. Les excepcions a aquesta tendència es van trobar a les estacions dominades per deposicions de sediments gruixuts. L'alta correlació entre la presència de cists i argiles durant els períodes d'absència de proliferació indica que els cists es comporten com partícules passives de sediment i que estan influenciats pels mateixos processos hidrodinàmics que les argiles. A Arenys de Mar, el principal forçament físic que afecta a la resuspensió de sediments és la seixa, que es va estudiar mitjançant mesures in situ i models numèrics per tal d'interpretar els patrons de distribució observats. Durant els períodes d'absència de proliferació, les pèrdues de cists van ser menors quan la seixa va ser més activa i a l'estació on les corrents induïdes per la seixa van ser majors. Per tant, la resuspensió forçada per la seixa sembla reduir les pèrdues de cists mitjançant la recol·locació dels cists de nou a la superfície del sediment evitant el seu soterrament en el sediment. Els perfils verticals d'abundància de cists observats van ser coherents amb aquest procés.

INTRODUCTION

A heteromorphic life cycle alternating between motile and resting stages is a common feature of many bloom-forming phytoplankton species (e.g., dinoflagellates, cyanobacteria, raphidophyceans). Shifts between these different life stages occur in response to endogenous and/or environmental factors and determine species survival, persistence, and spreading (Steidinger and Garcés, 2006). Since planktonic and benthic phases occupy distinct ecological niches, the factors controlling cell populations during each of these phases are different. For these reasons, it is essential to interpret phytoplankton proliferations by considering not only the motile vegetative phase of the species but also its complete life cycle as well as the factors influencing each stage.

In the case of dinoflagellates, the sexual phase of the life cycle includes a benthic resting cyst that results from a planozygote formed by the fusion of gametes. Cyst formation plays numerous roles in the ecology of dinoflagellates, including beneficial effects on species dispersal, genetic recombination, seeding for bloom initiation, and survival during unfavorable conditions (Dale, 1983). A thick protective wall improves the survival of resting cysts and facilitates their sinking to the sea bottom (Montresor et al., 1998). Indeed, cysts can rest in the sediments for decades, thus providing a reservoir of potential diversity (Belmonte et al., 1997).

Information about the fluctuations of resting cysts and the physical factors that determine their distribution is fundamental to understand and predict the onset and fate of phytoplankton blooms. Analyses of the temporal abundance of resting cysts are essential for the development of conceptual models of bloom dynamics, as underlined by the fact that cyst abundance in the sediment is considered to reflect the potential for subsequent blooms (Anderson et al., 2005; Villanoy et al., 2006). Additionally, surveys of sedimentary cyst assemblages may reveal the existence of species rarely observed in the plankton (Joyce, 2005; Satta et al., 2010), thereby providing an early indication of the presence of species of interest.

Among the factors known to influence the resting phase, bottom-boundary processes, and, more generally, the forces controlling sediment dynamics are highly relevant (Wang et al., 2004). Resting cysts tend to gather in zones where fine sediments accumulate, suggesting that dormant stages behave as passive particles in sediment dynamics (Dale, 1976). Dispersion and accumulation regulate the horizontal distribution of cysts, whereas burial, bioturbation by benthic organisms, and resuspension contribute to their vertical redistribution (Anderson et al., 1982; Giangrande et al., 2002). In addition to these processes, biotic factors, such as germination, natural mortality, degradation, and grazing, also determine cyst abundances in the sediment (Persson, 2000).

Reports of dinoflagellate outbreaks following periods of enhanced vertical mixing (e.g., Usup and Azanza, 1998) suggest a close linkage between resuspension and the early stages of bloom development. Anoxic conditions and the absence of light in the sediment repress cyst germination in most dinoflagellate species (Anderson et al., 1987), whereas wind forcing, strong tidal currents, and episodes of stormy weather, i.e., processes that cause resuspension, are likely triggers of excystment (Kirn et al., 2005; Kremp, 2001; Nehring, 1996). While in the open sea cyst abundance and species dominance patterns are the result of a complex combination of production, lateral transport, resuspension, and accumulation processes (Richter et al., 2007), in confined areas, where dispersion is restricted, the links between cysts and vegetative populations or particular environmental conditions are more straightforward (Garcés et al., 2004). Furthermore, confined areas play an important role as one-stop accumulation sites for cyst-forming species, allowing them to expand geographically.

As a result of their particular morphology (straight sidewalls, shallow and regular bathymetry, elongated configurations, etc.), harbors and other semi-enclosed systems are prone to natural oscillatory motions that occur within a time frame of one to several minutes (seiching). Although a certain degree of oscillatory movement is a feature of all enclosed basins, some harbors are subject to considerable seiches (e.g., Gomis et al., 1993). Resonance at these sites generates strong currents that resuspend sediments and other particulate material from the seafloor. Two major initiation forces are generally responsible for harbor resonance excitation: strong wind pulses directed to the closed end of the harbor and long-wave energy-loading from the sea and atmosphere (Lee and Park, 1998). While seiching is known to be relevant for the biology of limnic ecosystems (Ostrovsky et al., 1996), in the ocean it is most significant in confined areas, where tidal currents are low (microtidal regimes), and thus is likely to contribute to the triggering of near-shore algal blooms.

In this study, we determined the spatial and temporal distribution patterns of *Alexandrium minutum* Halim resting cysts and the sediment characteristics in Arenys de Mar harbor. *A. minutum* is a bloom-forming species able to trigger outbreaks of paralytic shellfish poisoning (PSP). It is widely distributed in the Mediterranean Sea (Vila et al., 2005 and references therein), including Arenys de Mar harbor, where recurrent blooms of *A. minutum* occur. In a previous study, Jordi et al. (2008) demonstrated, based on observations obtained by acoustic Doppler current profiler (ADCP) and numerical simulations, that seiching was the main physical process controlling the resuspension of harbor sediments. The present study combines *in situ* measurements with numerical modeling to explore the potential effects of seiche-forced resuspension on cyst distribution to provide evidence that the seiche regulates the distribution dynamics of the dormant phase of *A. minutum*. Moreover, the conclusions are in accordance with the results from an analysis of the vertical distribution of *A. minutum* resting cysts in the sediments.

METHODS

Study area

The study site was the harbor of Arenys de Mar, located on the coast of Catalonia (Spain, northwestern Mediterranean Sea, Fig. 1). The harbor is an artificial seaport measuring 0.4 km² and made up of shipyards, fishing docks, and a marina. The average depth is 4 m, ranging from 1 m in the most confined parts to 6 m at the entrance. The harbor receives both semi-continuous freshwater inflows along its walls and occasional discharges from land drainage following episodes of heavy rain. The tidal regime is microtidal, reaching a height of up to 0.25 m in spring-tide (Tsimplis et al., 1995). During the period of study, no dredging activities were carried out in the harbor. Water disturbance caused by ship traffic is very low and such activity consists mainly of leisure and small fishing boats.

Sampling strategy

Between the years 2005 and 2008, surveys were carried out in Arenys de Mar harbor in order to assess the spatial and temporal distribution patterns of *A. minutum* resting cysts and of the sediments. Surveys were conducted at different bloom conditions of the recurrent outbreaks of *A. minutum* vegetative cells that occurred in the harbor during that time. Data were obtained from 7–11 stations (Fig. 1) during five sediment surveys carried out before (C2 and C4, Fig. 2) and after (C1, C3, and C5, Fig. 2) the blooms. The exact dates of the surveys were informed by the numbers of *A. minutum* vegetative cells, obtained from the regional toxic phytoplankton monitoring program of Catalonia (station 23, Fig. 1). In addition, the abundance and spatial distribution of *A. minutum* vegetative cells during bloom development were determined by a survey performed at 14 stations on March 2006 (V, Fig. 2). Finally, to ascertain the vertical distribution of *A. minutum* resting cysts in sediments, a survey was carried out at three stations (23, 13, and 28, Fig. 1) prior to bloom development (C6, Fig. 2).

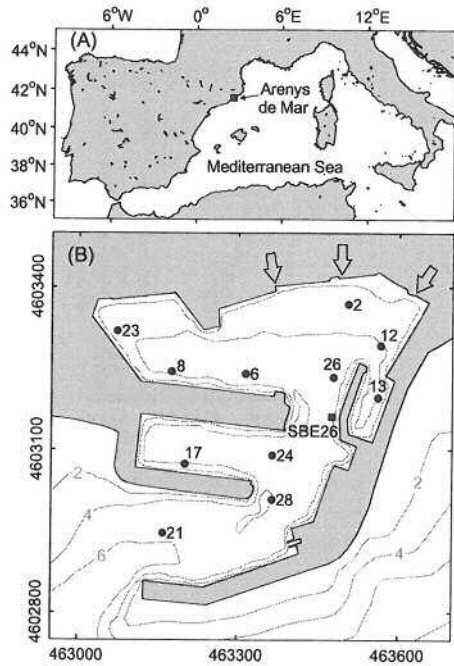


Fig. 1. (A) Location of Arenys de Mar. Latitude/Longitude coordinates in degrees. (B) Arenys de Mar harbor and the locations of the deployed instrument (square) and sampling stations (dots with station number). Arrows indicate drainage system inputs. Gray lines are isobaths (m). Coordinates are UTM (m).

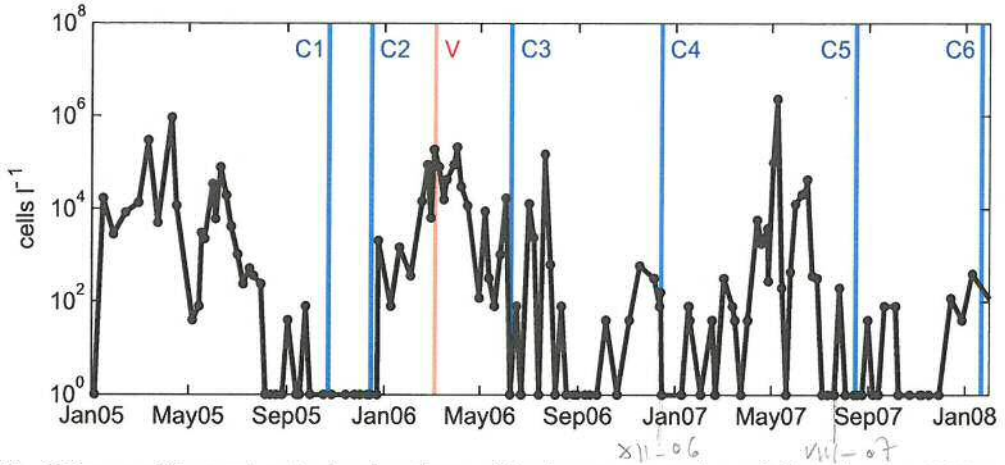


Fig. 2. Temporal fluctuations in the abundance of *A. minutum* vegetative cells from January 2005 to January 2008 (dots). Bars indicate surveys of resting cysts and sediments (C1–C5), vertical distribution of cysts (C6), and vegetative cells distribution during bloom development (V).

Sample collection, processing, and analysis

Phytoplankton samples to be evaluated in the monitoring program and the spatial survey were collected at the water surface at the same time of day (12:00 GMT) and fixed immediately with Lugol's solution. Vegetative cells were quantified by sedimentation of 50-ml subsamples in settling chambers for 24 h followed by counting of an appropriate area, depending on the density (Andersen and Throndsen, 2003) under an inverted microscope (Leica DMIRB bright-field and epifluorescence microscope). *A. minutum* cells were identified by staining with Calcofluor-White solution (Fritz and Triemer, 1985).

For sediment surveys, two samples per station were collected by a scuba diver, who inserted plastic cylindrical corers (20 cm long \times 5 cm base diameter) into the sediments. For resting cyst analysis, sediment cores were stored in the dark at 10°C and left to settle for at least 24 h. All of the cores from each survey were processed within one month. The water above the core was carefully removed and the sediment cut into 1-cm slices. A subsample of the top 0–1 cm (temporal and spatial distributions) and one from each 1-cm slice of the 0–5 cm layer (vertical distribution) were sonicated, sieved to retain the 10- to 100- μ m fraction, and processed by the sodium polytungstate density gradient method of Bolch (1997), as modified by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed in a 10- μ m sieve and collected with 10 ml of filtered seawater. *A. minutum* resting cysts were counted in 2-ml Utermöhl sedimentation chambers under an inverted microscope (see above specifications). Empty cysts were not considered, and abundance was expressed as cysts ml⁻¹ of wet sediment (ws). The cysts were almost hemispherical in shape when viewed directly and kidney shaped in lateral view, in accordance with the descriptions of Garcés et al. (2004) and Bravo et al. (2006). Further morphological details of *A. minutum* cysts identified in the harbor sediments can be found in Satta et al. (this issue).

Sediment characteristics were analyzed by measuring the grain size of the <50- μm fraction using a Sedigraph 5000D and of the >50- μm fraction using a settling tube, according to the method of Giró and Maldonado (1985). Granulometric distributions of both fractions were combined and total grain-size distribution, and textural statistical parameters for each sediment sample were calculated. Carbon was measured in duplicate using a Leco CN 2000 analyzer. Two subsamples were used to determine the total carbon percentage after combustion at 1050 °C. Another two subsamples were digested with HCl in a LECO CC 100 digester; the resulting CO₂ was measured using the same LECO CN 2000 analyzer and assigned to inorganic carbon content. The difference between the two values is the percentage of organic carbon (OC). Spatial objective analysis

Spatial maps of vegetative cells, resting cysts, and granulometry were interpolated using spatial objective analysis for each survey and an independent data set (Pedder, 1993). Since the presence of nearby sampling points separated by piers may have biased the analysis, the position of the coastline was taken into account in the interpolation in order to constrain the statistical error by computing the covariance of the marine domain only. This prevented propagation of information across the coast.

Seiche characterization

To determine the characteristics of the seiche in the Arenys de Mar harbor, an SBE26 sea-level recorder was deployed at the same location for two periods (September 22, 2005—July 26, 2006, and January 19—May 23, 2007), as shown in Figure 1. The original pressure–time series, recorded every 30 s, was adjusted to sea level with atmospheric pressure, measured at a nearby station (scale factor 1 cm mb⁻¹). Point measurements were compared and extended to the entire harbor domain using a three-dimensional finite-element model (FUNDY) based on the linear shallow-water equations. The algorithm uses conventional hydrostatic and Boussinesq approximations and Mellor-Yamada level 2.5 eddy viscosity closure. Details of the model are given in Lynch and Werner (1987) and Lynch et al. (1992). The computational domain extended from the harbor to the inner shelf. The mesh contained 4249 elements and 2428 nodes in the horizontal direction, with 11 one-dimensional linear elements connected under each horizontal node following a sigma coordinate system. The model was forced by a barotropic wave of 1-cm amplitude at the model boundaries, which lay far from the harbor and hence were unlikely to influence its response. Solutions were obtained in the frequency domain. Different simulations with wave periods ranging from 1 min to 5 h were performed. The relative amplification of waves arriving from the shelf inside the harbor was evaluated by dividing the amplitude of the sea-level response measured at the location of the sea-level recorder by the amplitude of the wave at the ocean boundaries.

Potential influence of the seiche and analysis of the vertical distribution of *A. minutum* cysts

To study the potential influence of the seiche on the vertical distribution of *A. minutum* resting cysts in Arenys de Mar harbor, a coupled hydrodynamic sediment-cyst one-dimensional vertical model was used to investigate the effect of resuspension events on the sediments and on resting cysts. The model was described in detail in Jordi et al. (2008) and included the simulation of two sediment classes, sands and fine sediments (the sum of silts and clays). Here, we introduced a new sediment class comprising sediments with a diameter of 20 μm and a density of 1250 kg m^{-3} (Anderson et al., 1985), thus representing *A. minutum* resting cysts. Biological processes, such as germination, mortality, and predation, were not simulated and resting cysts were treated as passive particles.

In addition, the vertical profiles of resting cysts were examined at three stations, each of which reflected different seiche effects. If we assume that seiche-induced mixing can be described as a diffusive process, then the vertical profiles obtained can be compared to the theoretical distribution as stated by the advection-diffusion equation (Guinasso and Schink, 1975):

$$\frac{\partial \rho C}{\partial t} = \frac{\partial}{\partial z} \left[D_b \frac{\partial \rho C}{\partial z} \right] - S \frac{\partial \rho C}{\partial z} \quad (1)$$

where C is the cyst concentration (cyst cm^{-3} ws), ρ the sediment density (g dry sediment cm^{-3} ws), t the time (s), z the depth (cm), D_b the sediment mixing rate ($\text{cm}^2 \text{s}^{-1}$), and S the sedimentation rate (cm s^{-1}).

In non-bloom periods, i.e., in the absence of cyst deposition, a steady state can be assumed for cysts in the sediment. If we also assume that the seiche-forced resuspension causes constant mixing in the sediment mixed layer (or in the depth scale of interest, 5 cm), the steady state solution of Eq. (1) becomes:

$$C = C_0 \exp\left(\frac{S}{D_b} z\right) \quad (2)$$

where C_0 is the cyst concentration at the sediment surface.

However, after the bloom cysts are deposited in the sediment, upsetting the steady state condition assumed in Eq. (2). If the arrival of a cyst at the sediment surface is considered as a pulse input caused by the bloom, then the solution of Eq. (1) is (Crank, 1975):

$$C = C_0 \exp\left(-\frac{z^2}{4D_b t}\right) \quad (3)$$

assuming that sedimentation was negligible ($S=0$) over the time interval (t) of interest. Therefore, differences in the slope of the exponential decrease in the number of cysts [the term between parentheses in Eq. (3)] can be explained by the sediment mixing rate (D_b).

RESULTS

Temporal abundances and spatial distribution of A. minutum vegetative cells

From January 2005 to January 2008, *A. minutum* blooms (up to 2×10^6 cells l^{-1}) were recurrently observed at Arenys de Mar harbor, generally over a period spanning late winter to early summer (Fig. 2). The spatial distribution of *A. minutum* vegetative cells during the maintenance phase of the 2006 bloom (V) is shown in Figure 3. Higher cell abundances were recorded at stations located in the innermost part of the harbor (1×10^5 cells l^{-1} at station 23), whereas the lowest abundances were measured at stations on the eastern side and towards the harbor entrance.

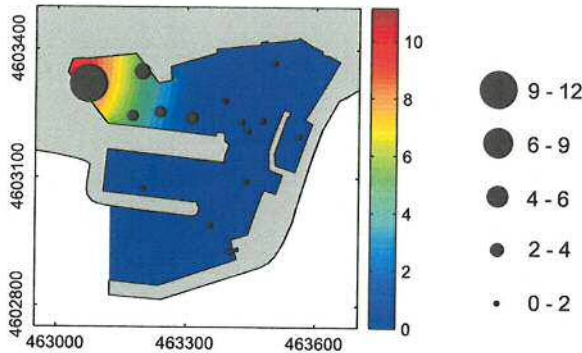


Fig. 3. Abundance of *A. minutum* vegetative cells (10^4 cells l^{-1}) in Arenys de Mar harbor during the maintenance bloom phase (2006). Data are presented as dots, with size related to cell abundance, and in colored maps from the interpolation of data by spatial objective analysis. Coordinates are UTM (m).

Temporal and spatial distribution patterns of A. minutum resting cysts

Abundances of *A. minutum* resting cysts during the period investigated ranged from 0 cysts ml^{-1} ws (C1, station 28) to 1010 cysts ml^{-1} ws (C5, station 23) (Fig. 4). Maximum values were always measured at the innermost part of the harbor (station 23). In some cases (C3 and C5), cysts also accumulated in the confined eastern basin (station 13). Cyst abundance was always lowest near the harbor entrance (stations 21 and 28). While the spatial distribution pattern of *A. minutum* resting cysts was fairly uniform throughout the different surveys,

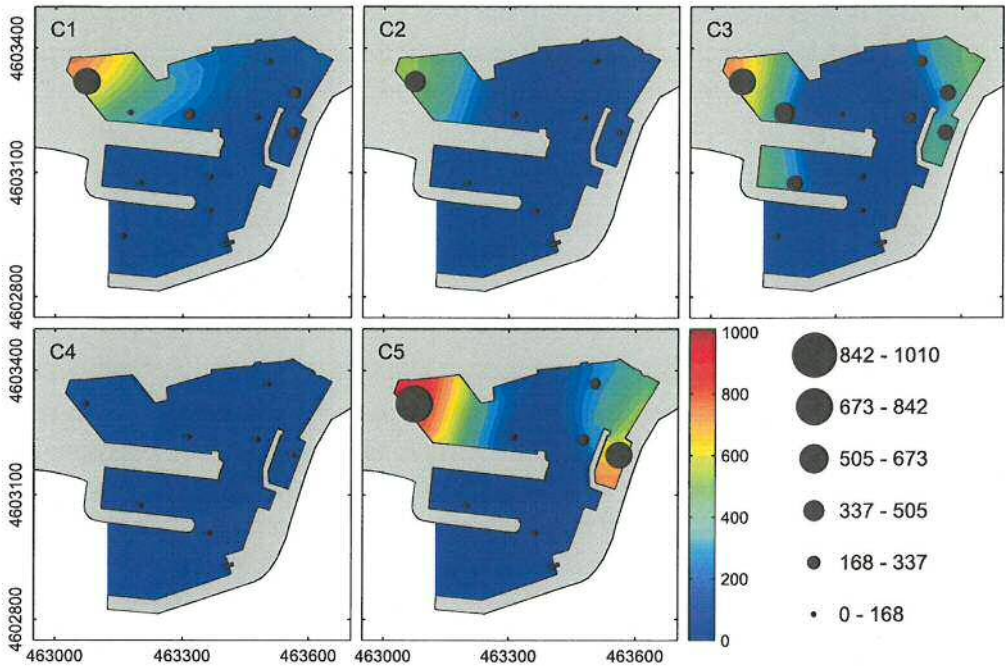


Fig. 4. Distribution and abundance of *A. minutum* resting cysts (cysts ml⁻¹ ws) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

there were large temporal variations in their abundance. A significant decreasing tendency in cyst abundance paralleled the absence of *A. minutum* blooms in the water column, while higher values were noted after the bloom, as a result of cyst production.

To evaluate gains and losses of resting cysts between bloom and non-bloom periods, the difference in total resting cyst abundances per station, as measured during two consecutive surveys, was calculated. The net balance for each period was expressed as the percent increase (positive numbers) or decrease (negative numbers) in resting cyst abundance during a given survey compared to the previous one (Table 1). The results showed a general trend in the variations of sedimented resting cysts in the harbor, with increases in cyst abundance after bloom periods and significant decreases after non-bloom periods. Exceptions occurred at some stations: an increase in resting cysts rather than a decrease was recorded at stations 17, 26, and 28 for the non-bloom period C1–C2, and a decrease in resting cysts was observed at station 6 during the post-bloom period C2–C3. However, since the resting cyst abundance measured at these stations is very low, these increases are not very meaningful. In that case, if these exceptions are excluded, cyst losses at the stations were more homogeneous throughout non-bloom periods than cyst gains occurring after blooms. The net balance of the post-bloom periods differed, with a higher increase during the C4–C5 period, whereas the extent of the decrease was greater for the non-bloom period of C3–C4.

Station	C1-C2 (non-bloom)	C2-C3 (bloom)	C3-C4 (non-bloom)	C4-C5 (bloom)
2	-56	348	-89	743
6	-58	-15	-9	41
13	-52	293	-84	1115
17	901	561	-94	347
23	-26	39	-80	525
26	982	86	-88	739
28	24	454	-59	99

Table 1. Net balance of gains and losses of resting cysts between non-bloom and bloom periods, expressed as the percent increase (positive numbers) or decrease (negative numbers) in resting cyst abundances during a given survey compared to the previous one. Only the seven stations common to all the resting cyst surveys are considered.

Temporal and spatial sediment patterns

The seafloor at Arenys de Mar is composed of fine-grained sediments with an average particle size of $14.6 \pm 12 \mu\text{m}$ (fine silt). Figures 5–7 show the spatial and temporal changes

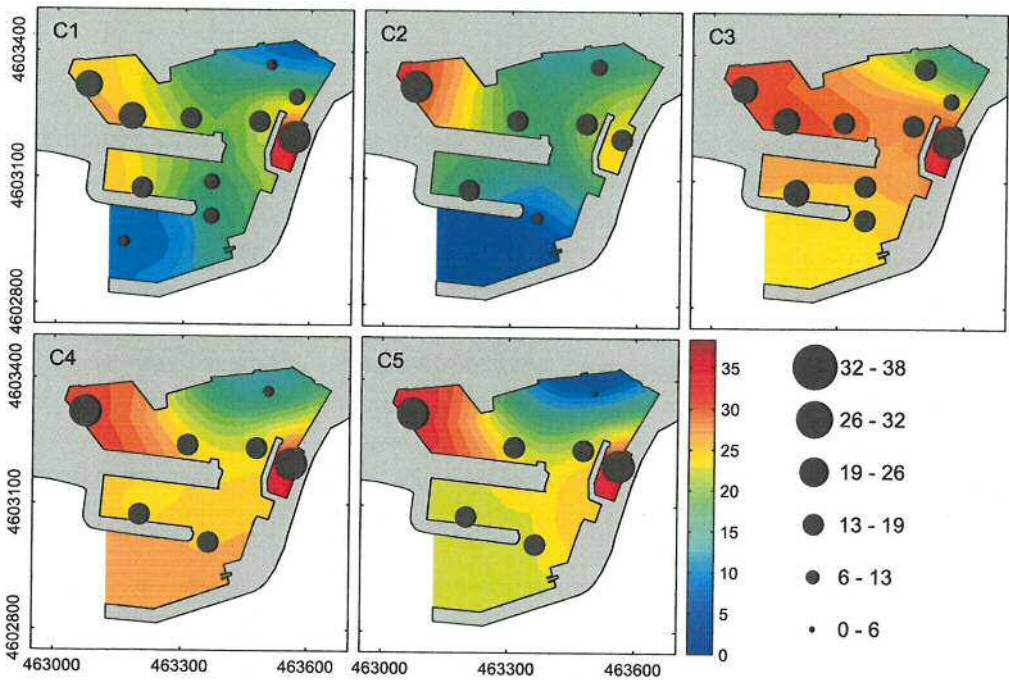


Fig. 5. Distribution of the clay fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

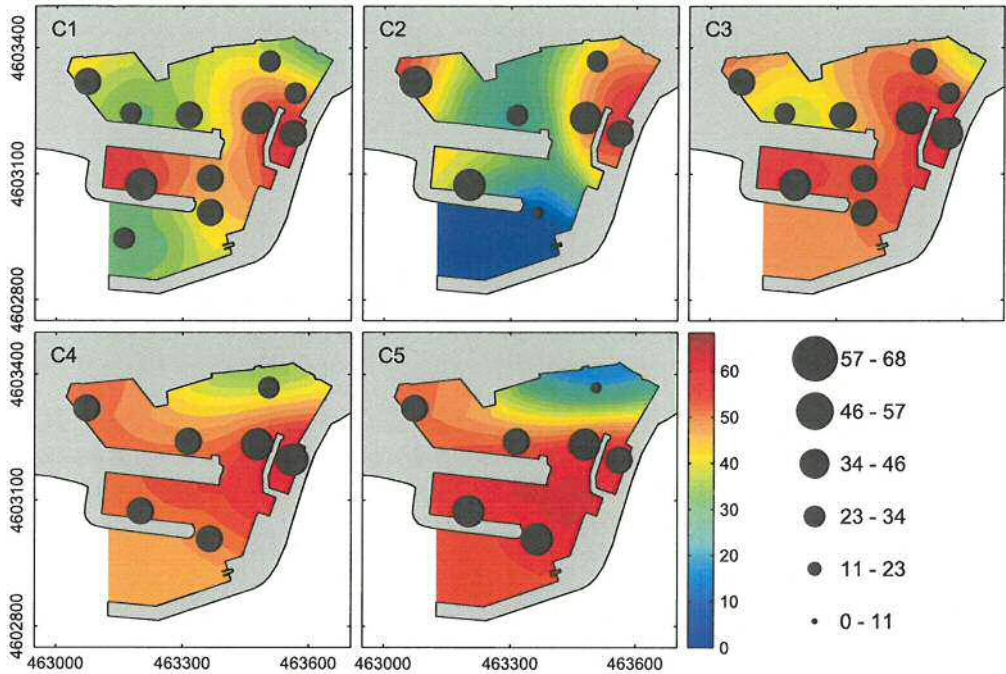


Fig. 6. Distribution of the silt fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

in the harbor's sediment fractions of clays, silts, and sands. Fine-sediment fractions (clay and silt) were the most abundant (average of 23% and 50%, respectively). A maximum of fine-sediment fractions frequently occurred in the inner basins of the harbor (stations 13, 17, and 23). Sands were less abundant than fine fractions, although there was noticeable variability, probably related to sediment inputs from external sources. As shown in Figure 7, these inputs affected the harbor entrance (C1 and C2), the northeast corner (station 2) where the drainage system ends (C1, C4, and C5), and the northern side of the main dock (station 6) where construction materials had on occasion been deposited (C1 and C2). The gravel-containing fraction accounted for only 0–3% of the sediments, with the highest values recorded at the harbor entrance (stations 21 and 28) and the northern side of the main dock (station 6). Therefore, these areas are prone to coarse sediment deposition. The OC content of the sediment ranged from 0.9% to 3%. OC content was higher in the inner basins of the harbor and had the same distribution pattern as fine-sediment fractions.

Correlations between resting cyst abundances and sediment fraction percentages showed that the highest correlation was between cyst concentrations and the percentage of clay ($R^2=0.43$), considering all data sets. A higher correlation coefficient ($R^2=0.89$) was obtained when this correlation was evaluated by considering only the data from pre-bloom surveys (C2 and C4).

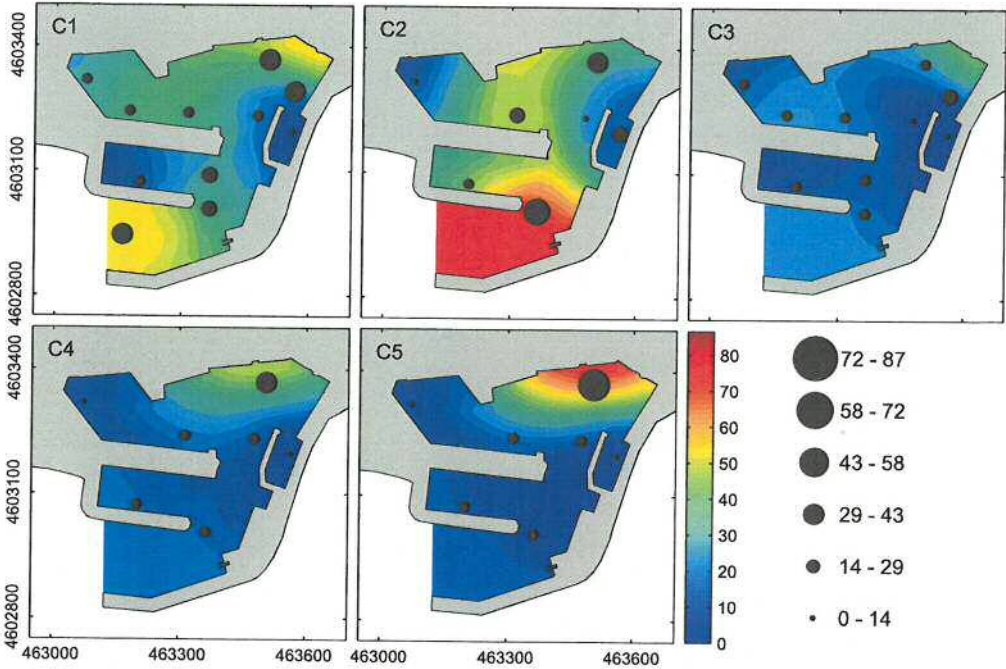


Fig 7. Distribution of the sand fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

Seiche characteristics

Sea-level spectra (Fig. 8A) for the two periods when the SBE26 sea-level recorder was deployed were estimated using a Hamming window of 512 points, with a half-window overlap (Emery and Thomson, 1997). The computed spectra for the two periods were very similar, although those for 2005–2006 were slightly more energetic, suggesting a more active seiche in that period than in 2007. The main feature of the spectra was a very energetic peak, with a period of 12.8 min, related to the fundamental (Helmholtz) mode of Arenys de Mar harbor. Two lower spectral peaks were also observed, at wave periods of 3.9 min and 2.7 min. The sea-level amplification for the harbor, computed at the sea-level recorder location using the numerical model, is shown in Figure 8B. The agreement in the position of the peaks between the measured spectra and the computed amplification is very reasonable, indicating that the model properly simulated the harbor response.

The spatial distribution of the fundamental resonant mode (12.8 min) obtained from the numerical analysis revealed large sea-level amplification in the inner part of the harbor and one node at the entrance (Fig. 9A), consistent with the theory of natural resonance phenomena. The spatial distribution of the second peak (3.9 min) had two nodes, one at

the entrance and the other at a distance of approximately two-thirds of the harbor's length, as measured from the entrance (Fig. 9B). These corresponded to the first mode of Arenys de Mar harbor whereas the lower spectral peak (2.7 min) reflected a more complicated spatial distribution and corresponded to the second mode (not shown). Similar to the sea-level amplification, currents associated with the harbor response differed significantly from site to site according to their position relative to the oscillation nodes. Jordi et al. (2008) analyzed seiche-driven current variability and associated resuspension events at a location near the SBE26. Here, we used the numerical model to extend their results to our sampling stations.

Figure 10 shows the current amplification at stations 23, 13, and 28, where the vertical distribution of cysts was analyzed, and at station SBE26 for a comparison with the results of Jordi et al. (2008). In the fundamental mode, the currents were highly amplified at the harbor entrance (station 28), whereas those in the first mode

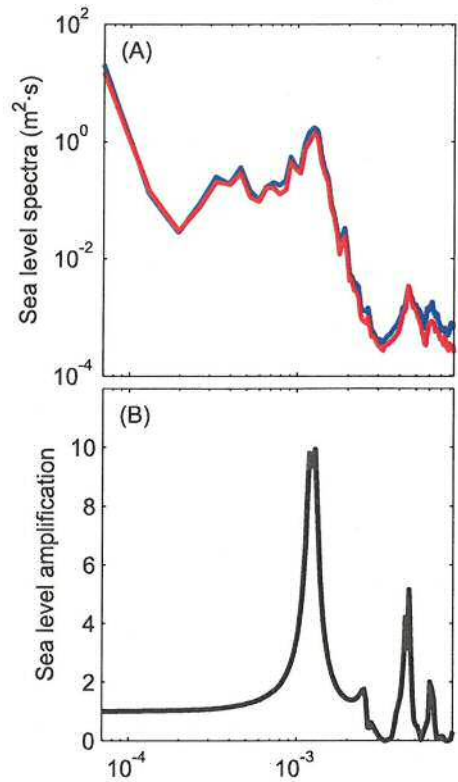


Fig. 8. (A) Sea-level spectral density at the SBE26 location. (B) Sea-level amplification computed with the numerical model at the SBE26 location.

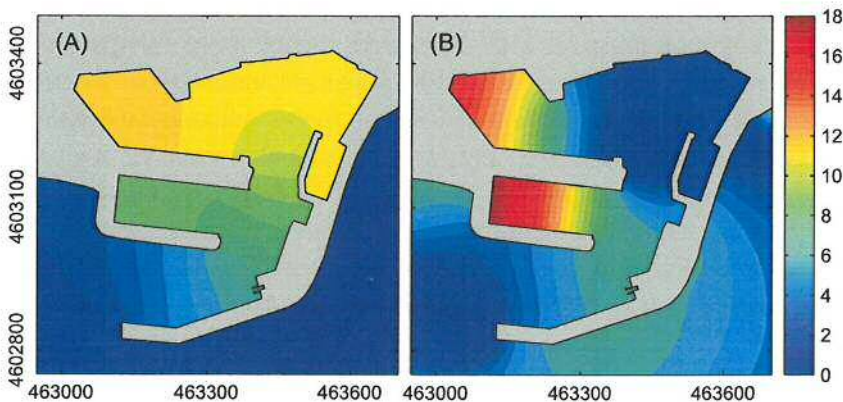


Fig. 9. Spatial distribution of the harbor response (relative sea-level amplification) for (A) the fundamental mode, and (B) the first mode. Coordinates are UTM (m).

were significantly larger in the inner basin (station 23). The currents at SBE26 were strong enough to resuspend the sediment (Jordi et al., 2008); accordingly, those at stations 28 and 23 must be able to do so as well. In contrast, the seiche had little effect on currents inside the confined eastern basin (station 13).

Influence of the seiche on the distribution of A. minutum resting cysts

Since clays are the sediment fraction with the highest correlation to resting cysts, only the resuspension of these two classes of particles was analyzed in the one-dimensional vertical model simulations. Figure 11 shows the simulated resuspension in response to a seiche event 10 cm in amplitude and 6 h in duration, i.e., the usual conditions of a strong event in the Arenys de Mar harbor. Resting cysts undergo a higher degree of resuspension than clays due to their lower density. In addition, they remain resuspended in the water column for a longer (about eight times) period of

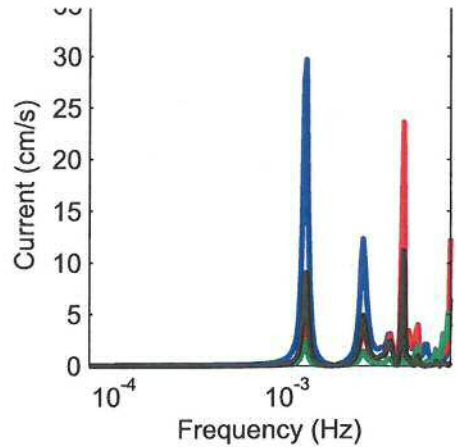


Fig. 10. Currents induced by waves of different frequencies at stations 23 (red), 13 (green), and 28 (blue) and at station SBE26 (black).

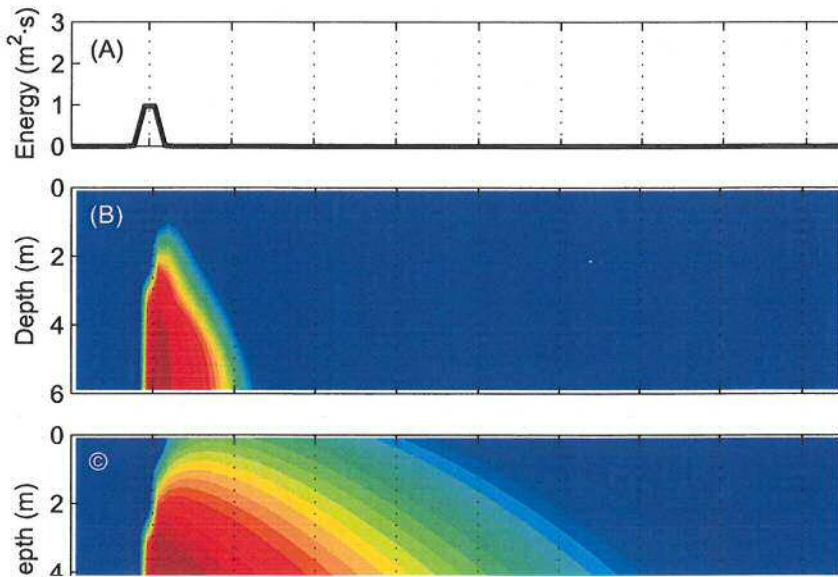


Fig. 11. (A) Energy of a seiche event 10 cm in amplitude and 6 h in duration. (B) Simulation of the seiche-forced resuspension of clays. (C) Simulation of the seiche forced resuspension of resting cysts.

time and are deposited in the surface sediment later than clays. This implies sediment mixing and reallocation of the cysts to the sediment surface.

The vertical distributions of *A. minutum* resting cysts at the selected stations are shown in Figure 12. Surface maxima as well as lower abundances below the surface were recorded at stations 23 and 13. The surface abundances at the two stations were similar, but while there was a progressive decrease with depth at station 23, the abundances remained relatively homogeneous at station 13, where a subsurface peak was detected at a depth of 4 cm. Low, homogeneously distributed cyst abundances were measured throughout the profile at station 28. Since the vertical profiles were obtained prior to development of the bloom, a steady state was assumed and the profiles could be explained by Eq. (2). To test the validity of this equation, the cyst profiles were subjected to an exponential by least-squares, as shown in Figure 12. The highest correlation was found at station 23, and the lowest at station 28.

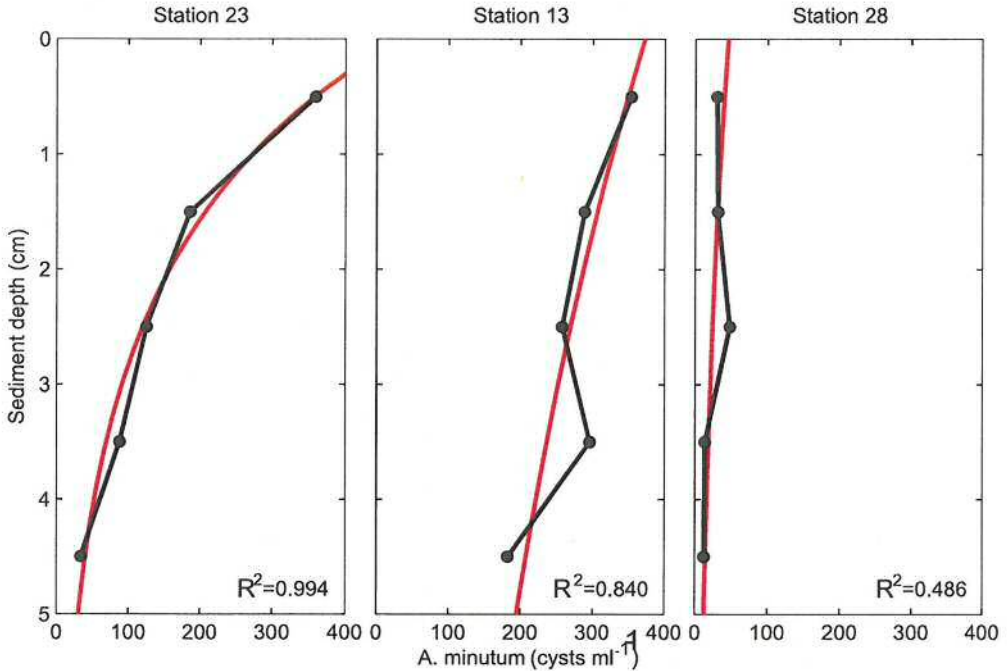


Fig. 12. Vertical profiles of the abundances of *A. minutum* resting cysts (dotted line) and the exponential fit (solid line) at three stations representative of the different seiche effects.

DISCUSSION

The present study evaluated the fate of *A. minutum* resting cysts in the sedimentary regime over three bloom cycles. The spatial and temporal distributions of the resting cysts and of the sediments in the Arenys de Mar harbor were investigated throughout different stages of the vegetative cells bloom. A numerical model was used to assess the distribution of seiche motion and associated currents in the harbor. The following discussion analyzes the distribution dynamics of resting cysts with respect to physical forcing in Arenys de Mar harbor, particularly seiche motion. Jordi et al. (2008) identified seiching as the physical forcing that controls sediment resuspension in the harbor, whereas neither tides, nor wind-driven currents, nor waves are strong enough to resuspend the bottom sediment. They also reported sporadic events of sediment advection from outside waters and freshwater discharges from land-based sources.

Changes in the abundance of cysts present in surface sediments are the net result of biological and physical processes acting on the seedbank (Anderson et al., 1982). Biological processes include benthic animal activity and species-specific characteristics, such as cyst deposition, germination, and mortality, while physical processes include mixing, sedimentation, and resuspension (Keafer et al., 1992). Our data suggest that, immediately after the bloom of vegetative cells, the distribution and abundance of *A. minutum* resting cysts depend mainly on the biological process of cyst formation and cyst deposition. First, the correlation between resting cyst abundances and clay sediment fraction percentage significantly improved (from $R^2=0.43$ to $R^2=0.89$) when values of post-bloom surveys were excluded, indicating that cysts are not influenced by processes related to the sediment dynamics at this time of the bloom. Correlations between cysts and sediments need to be interpreted taking into account the period of bloom and the recent cyst deposition. Second, variations recorded over the monitored period showed that resting cyst abundance in the sediment increased substantially after the bloom, suggesting that the horizontal distribution of resting cysts and their respective abundance are a reflection of cyst production. As spatial variations in deposition are the more likely cause of interannual variations in cyst abundance (Anderson et al., 2005), the variability in the increases in cyst abundance after bloom periods was probably due to heterogeneous cyst deposition, determined in large part by the spatial distribution of vegetative cells during cyst production. Indeed, in the post-bloom survey in 2006, resting cyst abundances were highest under the overlying vegetative cell maximum recorded during bloom development (Fig. 3), as Garcés et al. (2004) observed in a cyst survey performed after the bloom in 2002. The differences in total resting cyst abundance observed between the two bloom periods investigated here provide additional evidence for the importance of the biological process of cyst formation. These variations could be related to an unequal efficiency in cyst production during the two bloom periods. Further studies should be carried out to test this hypothesis. In non-bloom periods, the pool of resting cysts decreases due to germination, mortality, and predation, together with the lack of deposition of new

cysts in the absence of vegetative cells in the water column. The homogeneous decreases measured at nearly all stations indicated that similar losses occurred throughout the harbor. Furthermore, the high correlation between cysts and clays during non-bloom periods confirmed that resting cysts behave as passive particles. Consequently, they are subject to resuspension and thus can be transported by water currents, which favors their dispersal to new areas. In Arenys de Mar harbor, oscillatory currents associated with the seiche are able to resuspend sediments and cysts (Fig. 11), but they transport sediments over rather short distances (Jordi et al., 2008). Other processes, such as wind-driven currents and sediment advection, are likely to be more effective in redistributing suspended sediments and cysts over longer distances. Van Lenning et al. (2007) showed that wind-driven currents favored the accumulation of *A. minutum* vegetative cells in the northeastern region of the harbor during the bloom in 2003, and it is expected that similar currents transport suspended cysts. Sediment advection, although a less-frequent occurrence (Jordi et al., 2008), is also likely to redistribute cysts in the harbor. In fact, exceptions to the general trend of cyst increases and decreases were noted in areas affected by the deposition of coarse sediments, which, in turn, is linked to sediment inputs entering the harbor system. Cysts deposited in the seabed may eventually mix with sediments, which may at least in part explain the observed decreases in cyst abundance. Cyst redistribution by benthic animals is not significant in Arenys de Mar (Garcés et al., 2004); instead, sediment mixing processes are most probably driven by seiche-forced resuspension. Sporadic sediment inputs from external sources can cover sedimented cysts, which are then reallocated to the sediment surface by seiche-forced resuspension, thus tending to reduce decreases in abundance. This is supported by the fact that cyst losses were greater in 2007 (C3–C4 period), when the seiche was less active, than in 2006 (C1–C2 period). However, the time span between the C3 and C4 surveys was longer than that between C1 and C2, which would also account for the greater losses.

In this context, stations 23 and 13 were chosen to study the effects of seiche on the vertical profiles of cyst abundance because they are less influenced by sediment inputs entering the harbor, in contrast to station 28, where there are sand inputs. At the sediment surface, cyst decreases during non-bloom periods were found to be smaller at station 23, where the seiche-induced currents were larger than those at station 13. As is the case for spatial and temporal patterns of cyst abundance, the vertical profiles represent the net balance of gains and losses of surface and subsurface cyst concentrations. Results of the least-squares analysis of *A. minutum* resting cyst profiles (Fig. 12), carried out to test the validity of Eq. (2), showed that the correlation between the exponential fit and the measured cyst abundance was highest at station 23, where the seiche-induced currents are relatively large and coarse sediments are few. At station 13, however, the seiche-induced currents are very low such that the depth of the mixed layer is likely reduced to less than 5 cm, which explains the poor correlation obtained at this station. In addition, sediment deposition can redistribute cysts to depths below the mixed layer. Considering station 28, although the seiche-induced currents at this station are large and should have resulted in strong

mixing, the correlation between the measured cyst abundance and the exponential fit was low due to the deposition of sands, which may cover sedimented cysts and therefore inhibit their resuspension and mixing. Consequently, the homogeneous profile was similar to that recorded at station 13. Similar vertical distributions of cysts were reported by Giannakourou et al. (2005), who found that significant resuspension contributed to the mixing of the upper sediment layers, resulting in more homogeneous cyst profiles and less-pronounced subsurface peaks. After resuspension, homogeneous profiles were more evident at stations with higher sand-fraction percentages, and the presence of subsurface peaks was related to stations where the rate of sediment deposition is high.

With respect to post-bloom periods, Garcés et al. (2004) studied the vertical distribution of *A. minutum* resting cysts in the sediment of Arenys de Mar harbor after the bloom in 2002. Their profiles showed a peak at the surface that decreased notably with sediment depth, similar to the shape predicted by Eq. (2). However, since the cyst profiles were taken after the bloom, they are instead described by Eq. (3), in which differences in the slope of the exponential decrease in cyst number are a result of the sediment mixing rate (D_b). If Eq. (3) is fitted by least squares to the cyst profiles of Garcés et al. (2004), lower slope terms (larger mixing) are obtained at their stations 1, 2, 3, and 4, which were geographically closer to stations 21, 26, 17, and 8 of this study, respectively. By contrast, the slope values were larger (lower mixing) at stations 5, 6, 7, and 8 of their study (closer to our stations 13, 12, and 2). This finding of larger and lower mixing coincides with those areas characterized by larger and lower seiche-induced currents and supports the conclusion that seiching is the main factor controlling the vertical distribution of cysts in Arenys de Mar harbor.

Resuspension not only reallocates resting cysts back to the sediment surface, but also transports resting cysts to the water column, where conditions may be more favorable for germination and growth (Kirn et al., 2005). Vertical simulations of the resuspension of resting cysts and clays showed that dormant stages remain suspended in the water column about eight times longer than sediments. This would expose the cysts to temperature, light, and oxygen conditions that better promote germination compared to the conditions at the sediment surface. In addition, the suspension of cysts may significantly reduce the bloom initiation time compared to germination occurring exclusively from the sediment surface (Nehring, 1996). Furthermore, another effect of resuspension is the release of substances such as nutrients to the water, which stimulates phytoplankton growth (Rengefors et al., 2004). Hence, resuspension not only plays a key role in the distribution of resting cysts, but also could favor the germination of resting cysts and the growth of vegetative cells, giving rise to bloom development.

CONCLUSIONS

The spatial and temporal distribution patterns of *A. minutum* resting cysts in Arenys de Mar harbor together with variations in cyst abundance suggest that the abundance of resting cysts after bloom periods mainly depends on the biological process of cyst formation (spatial distribution of vegetative cells and magnitude of resting cyst production). Once deposited, cysts mix with the sediments, where they are influenced by the same hydrodynamic factors that govern the sediments. Seiche, the physical process that controls sediment resuspension in the harbor, also regulates the distribution dynamics of the dormant phase of *A. minutum*. Decreases in cyst abundances during non-bloom periods, in the absence of cyst production, are determined mostly by seiche-forced resuspension and the sporadic events of coarse sediment deposition. Resuspension, as suggested by the vertical profiles of the cysts, tends to reduce cyst losses by reallocating buried cysts to the sediment surface. Seiche-forced resuspension is therefore an important mechanism regulating the distribution and abundance of resting cysts in microtidal or semi-enclosed systems such as harbors or lakes. This may also be the case in more open areas, where resuspension arises from other major physical forcing, i.e., tides, currents, and/or waves. As resuspension could favor the germination of resting cysts and the growth of vegetative cells, further investigation should be addressed to elucidate its possible role in triggering phytoplankton blooms.

ACKNOWLEDGEMENTS

The authors thank N. Sampedro and A. Reñé for data from the toxic phytoplankton monitoring program, and S. de Diago and N. Maestro for the sediment analysis. B. Casas, K. Van Lenning and X. Novell provided valuable help with the fieldwork. We also are grateful to Creu Roja, Club Nàutic and Cofradia de Pescadors St Telm of Arenys de Mar harbor. This study was financed by the EC-funded Research Project SEED (GOCE-CT-2005-003875). The work of A. Jordi and E. Garcés was supported by a postdoctoral grant and a Ramon y Cajal award, respectively, both from the Spanish Ministry of Science and Innovation.

REFERENCES

- Amorim, A., Dale, B., Godinho, R., Brotas, V., 2001. *Gymnodinium catenatum*-like cysts (Dinophyceae) in recent sediments from the coast of Portugal. *Phycologia* 40, 572-582.
- Andersen, P., Thronsen, J., 2003. Estimating cell numbers. In: Hallegraeff, G.M., Anderson, D.M., Cembella, D.A. (Eds.), *Manual on harmful marine microalgae*. UNESCO Publishing, Paris, pp. 99-129.
- Anderson, D.M., Aubrey, D.G., Tyler, M.A., Coats, D.W., 1982. Vertical and horizontal distributions of dinoflagellate cysts in sediments. *Limnology and Oceanography* 27, 757-765.
- Anderson, D.M., Taylor, C.D., Armbrust, E.V., 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. *Limnology and Oceanography* 32, 340-351.
- Anderson, D.M., Stock, C.A., Keafer, B.A., Nelson, A.B., Thompson, B., McGillicuddy, D.J., Keller, M., Matrai, P.A., Martin, J., 2005. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. *Deep-Sea Research Part II-Topical Studies in Oceanography* 52, 2522-2542.
- Belmonte, G., Miglietta, A., Rubino, F., Boero, F., 1997. Morphological convergence of resting stages of planktonic organisms: a review. *Hydrobiologia* 355, 159-165.
- Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. *Phycologia* 36, 472-478.
- Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. *European Journal of Phycology* 41, 293-302.
- Crank, J., 1975. *The Mathematics of Diffusion*. Oxford University Press, Oxford.
- Dale, B., 1976. Cyst formation, sedimentation, and preservation: Factors affecting dinoflagellate assemblages in recent sediments from Trondheims Fjord, Norway. *Review of Palaeobotany and Palynology* 22, 39-60.
- Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (Ed.), *Survival Strategies of the Algae*. Cambridge Univ. Press, pp. 69-136.
- Emery, W.J., Thomson, R.E., 1997. *Data Analysis Methods in Physical Oceanography*. Pergamon, New York.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcofluor white M2R for the visualization of dinoflagellate thecal plates. *Journal of Phycology* 21, 662-664.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). *Journal of Plankton Research* 26, 637-645.

Giangrande, A., Montresor, M., Cavallo, A., Licciano, M., 2002. Influence of *Naineris laevigata* (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. *Journal of Sea Research* 47, 97-108.

Giannakourou, A., Orlova, T.Y., Assimakopoulou, G., Pagou, K., 2005. Dinoflagellate cysts in recent marine sediments from Thermaikos Gulf, Greece: Effects of resuspension events on vertical cyst distribution. *Continental Shelf Research* 25, 2585-2596.

Giró, S., Maldonado, A., 1985. Análisis granulométrico por métodos automáticos: tubo de sedimentación y Sedigraph. *Acta Geológica Hispánica* 20, 95-102.

Gomis, D., Monserrat, S., Tintore, J., 1993. Pressure-forced seiches of large amplitude in inlets of the Balearic Islands. *Journal of Geophysical Research-Oceans* 98, 14437-14445.

Guinasso, N.L., Schink, D.R., 1975. Quantitative estimates of biological mixing rates in abyssal sediments. *Journal of Geophysical Research-Oceans and Atmospheres* 80, 3032-3043.

Jordi, A., Basterretxea, G., Casas, B., Anglès, S., Garcés, E., 2008. Seiche-forced resuspension events in a Mediterranean harbour. *Continental Shelf Research* 28, 505-515.

Joyce, L., 2005. Dinoflagellate cysts from surface sediments of Saldanha Bay, South Africa: an indication of the potential risk of harmful algal blooms. *Harmful Algae* 4, 309-318.

Keafer, B.A., Buesseler, K.O., Anderson, D.M., 1992. Burial of living dinoflagellate cysts in estuarine and nearshore sediments. *Marine Micropaleontology* 20, 147-161.

Kirn, S.L., Townsend, D.W., Pettigrew, N.R., 2005. Suspended *Alexandrium* spp. hypnozygote cysts in the Gulf of Maine. *Deep-Sea Research Part II-Topical Studies in Oceanography* 52, 2543-2559.

Kremp, A., 2001. Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea. *Marine Ecology Progress Series* 216, 57-66.

Lynch, D.R., Werner, F.E., 1987. 3-dimensional hydrodynamics on finite-elements. Part 1. Linearized Harmonic Model. *International Journal for Numerical Methods in Fluids* 7, 871-909.

Lynch, D.R., Werner, F.E., Greenberg, D.A., Loder, J.W., 1992. Diagnostic model for baroclinic, wind-driven and tidal circulation in shallow seas. *Continental Shelf Research* 12, 37-64.

Montesor, M., Zingone, A., Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. *Journal of Plankton Research* 20, 2291-2312.

Nehring, S., 1996. Recruitment of planktonic dinoflagellates: Importance of benthic resting stages and resuspension events. *Internationale Revue Der Gesamten Hydrobiologie* 81, 513-527.

Ostrovsky, I., Yacobi, Y.Z., Walline, P., Kalikhman, I., 1996. Seiche-induced mixing: Its impact on lake productivity. *Limnology and Oceanography* 41, 323-332.

Persson, A., 2000. Possible predation of cysts - a gap in the knowledge of dinoflagellate ecology?. *Journal of Plankton Research* 22, 803-809.

Richter, D., Vink, A., Zonneveld, K.A.F., Kuhlmann, H., Willems, H., 2007. Calcareous dinoflagellate cyst distributions in surface sediments from upwelling areas off NW Africa, and their relationships with environmental parameters of the upper water column. *Marine Micropaleontology* 63, 201-228.

Satta, C.T., Anglès, S., Garcés, E., Lugliè, A., Padedda, B.M., Sechi, N., 2010. Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. *Deep-Sea Research Part II-Topical Studies in Oceanography* 57, 256-267.

Steidinger, K.A., Garcés, E., 2006. Importance of Life Cycles in the Ecology of Harmful Algae. In: Graneli, E., Turner, J.T. (Eds.), *Ecology of Harmful Algae*. Springer-Verlag, Berlin Heidelberg, pp. 37-49.

Tsimplis, M.N., Proctor, R., Flather, R.A., 1995. A 2-dimensional tidal model for the Mediterranean Sea. *Journal of Geophysical Research-Oceans* 100, 16223-16239.

Usup, G., Azanza, R.V., 1998. Physiology and bloom dynamics of the tropical dinoflagellate *Pyrodinium bahamense*. In: Anderson, D.M., Cembella, A., Hallegraeff, G.M. (Eds.), *Physiological Ecology of Harmful Algal Blooms*. Springer-Verlag, Berlin, pp. 81-94.

Van Lenning, K., Vila, M., Masó, M., Garcés, E., Anglès, S., Sampedro, N., Morales-Blake, A., Camp, J., 2007. Short-term variations in development of a recurrent toxic *Alexandrium minutum*-dominated dinoflagellate bloom induced by meteorological conditions. *Journal of Phycology* 43, 892-907.

Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. *Harmful Algae* 4, 673-695.

Villanoy, C.L., Azanza, R.V., Altemerano, A., Casil, A.L., 2006. Attempts to model the bloom dynamics of *Pyrodinium*, a tropical toxic dinoflagellate. *Harmful Algae* 5, 156-183.

Wang, Z., Qi, Y., Lu, S., Wang, Y., Matsuoka, K., 2004. Seasonal distribution of dinoflagellate resting cysts in surface sediments from Changjiang River Estuary. *Phycological Research* 52, 387-395.